American Journal of Epidemiology

Vol. 160, No. 1
Printed in U.S.A.
DOI: 10.1093/aje/kwh170

Dietary Protein and the Risk of Cholecystectomy in a Cohort of US Women

The Nurses' Health Study

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Received for publication September 10, 2003; accepted for publication January 21, 2004.

In animals, vegetable protein can inhibit gallstone formation. Epidemiologic studies of dietary protein in relation to gallstone disease are sparse, and the effects of dietary protein of different origins are not clear. The authors aimed to examine the relation between dietary protein intake and risk of cholecystectomy among participants in the Nurses' Health Study, a cohort study of US women in 11 states. During 20 years of follow-up (1980–2000), the authors documented 7,831 cases of cholecystectomy. After adjustment for age, other known or suspected risk factors, and specific fats in a multivariate model, the relative risk of cholecystectomy for women in the highest quintile of dietary total protein intake compared with women in the lowest quintile was 1.00 (95% confidence interval (CI): 0.93, 1.08; p for trend = 0.46). When extreme quintiles were compared, the relative risk for animal protein intake was 1.07 (95% CI: 0.98, 1.15; p for trend = 0.08), whereas the relative risk for vegetable protein intake was 0.79 (95% CI: 0.71, 0.88; p for trend < 0.0001), with a significant dose-response relation. Additional mutual adjustment between animal and vegetable proteins did not materially alter the risks. These results suggest that increased consumption of vegetable protein in the context of an energy-balanced diet can reduce the risk of cholecystectomy in women.

cholecystectomy; diet; dietary proteins; gallstones; risk; women

Abbreviation: CI, confidence interval.

Gallstone disease is common among adults in Western countries (1), and it is increasingly a major cause of abdominal morbidity leading to hospital admission (2, 3). Among Western populations, an estimated 80 percent of gallstones are cholesterol stones. Although the pathogenesis of cholesterol gallstones is complex and involves multiple recognized steps, precipitation of cholesterol crystals from supersaturated bile is the determinant step in the genesis of cholesterol-rich stones (4). High plasma triglyceride levels and low plasma high density lipoprotein cholesterol levels are associated with greater risk of gallstone disease in clinical studies (4). In controlled feeding studies in humans, substitution of dietary protein for carbohydrate significantly increases high density lipoprotein cholesterol concentrations and decreases triglyceride concentrations (5, 6). In animals, dietary proteins affect biliary cholesterol concentration and precipitation and gallstone formation (7–10). In specific studies, both biliary lithogenic index and cholesterol levels decreased significantly with increasing levels of vegetable protein intake (8), and dietary vegetable protein markedly reduced the formation of cholesterol gallstones (9). In addition, pigs fed on a vegetable protein diet had a lower cholesterol precipitation rate than pigs fed on an animal protein diet (10).

Despite the biologic plausibility that dietary vegetable protein intake may reduce gallstone disease, epidemiologic data on the association between dietary protein and risk of gallstones are sparse and inconclusive (11–13). Studies have been limited by small numbers of subjects, incomplete measures of diet, or short-term assessment of diet. To address these issues, we used a validated, self-administered food frequency questionnaire to assess dietary intake of

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TABLE 1. Baseline characteristics of US women according to quintile of energy-adjusted intake of animal protein, Nurses' Health Study, 1980

Characteristic	Quintile of animal protein intake				
	1 (lowest)	2	3	4	5 (highest)
Median intake (g/day)	41.1	52.7	60.2	68.1	84.5
Mean age (years)	45.6	45.6	45.7	45.7	45.9
Current smoking (%)	32.4	28.9	27.4	27.2	25.7
Body mass index*	23.5	23.7	23.9	24.2	24.9
Physical activity (hours/week)	2.8	3.0	3.0	3.1	3.2
Any weight loss in prior 2 years (%)	27.8	27.1	28.1	28.9	34.8
Total energy intake (kcal/day)	1,549	1,581	1,586	1,584	1,553
Oral contraceptive use (%)	49.0	50.4	51.7	51.6	51.0
Use of hormone replacement therapy† (%)	12.6	13.1	13.7	14.2	14.5
Use of aspirin (%)	46.4	46.0	46.3	45.5	46.6
Use of thiazide diuretics (%)	6.9	7.4	7.5	8.7	10.2
Alcohol intake (g/day)	8.6	7.1	6.4	5.9	4.5
Caffeine intake (g/day)	404	399	391	387	382
Dietary intake (g/day)					
Saturated fat	24.0	27.2	28.8	30.1	29.8
Trans fat	4.1	4.2	4.1	4.0	3.6
Polyunsaturated fat	10.2	9.7	9.4	9.0	8.6
Monounsaturated fat	24.9	28.0	29.6	30.8	30.0
Carbohydrate	184	164	153	142	132
Fiber	14.3	13.3	12.9	12.6	12.7
Foods consumed (no. of servings/day)					
Red meat	1.0	1.3	1.5	1.6	1.5
Chicken and turkey	0.2	0.2	0.2	0.3	0.4
Fish	0.1	0.1	0.1	0.2	0.3
Fruits	2.2	2.1	2.1	2.0	2.0
Vegetables	1.7	1.8	1.9	2.0	2.2

* Weight (kg)/height (m)^2.
† Only postmenopausal women were included.

protein and other nutrients in the Nurses' Health Study (14). Estimates of protein intake were based on repeated dietary measurements taken during 20 years of follow-up, which provided us with a measure of long-term consumption of dietary protein. We examined the associations between intake of total protein and protein of animal and vegetable origin and the incidence of cholecystectomy in this large cohort of US women.

MATERIALS AND METHODS

Subjects

The Nurses' Health Study cohort was created in 1976 when 121,700 predominantly Caucasian female registered nurses aged 30–55 years who resided in 11 large US states completed a mailed questionnaire on their medical history and lifestyle. Every 2 years, follow-up questionnaires are sent to participants to obtain updated information on exposures and to identify newly diagnosed illnesses. In 1980, a 61-item food frequency questionnaire was included for assessment of intake of specific fats and other nutrients. In 1984, the food frequency questionnaire was expanded to include 116 items. After up to four mailings, 98,462 women returned the 1980 diet questionnaire. For the present analysis, we excluded women with implausibly high or low energy intakes (i.e., <500 kcal/day or >3,500 kcal/day), 11 or more blank food questionnaire items, or prior diagnosis of gallstone disease or cancer. The final baseline population consisted of 80,898 women. On average, more than 90 percent of participants responded to each subsequent biennial questionnaire, and approximately 80 percent completed each repeated dietary questionnaire during the follow-up period (14). This study was approved by the Brigham and Women's Hospital (Boston, Massachusetts) institutional review board on the use of human subjects in research.

TABLE 2. Baseline characteristics of US women according to quintile of energy-adjusted intake of vegetable protein, Nurses' Health Study, 1980

Characteristic	Quintile of vegetable protein intake				
	1 (lowest)	2	3	4	5 (highest)
Median intake (g/day)	9.1	11.9	13.9	16.1	21.0
Mean age (years)	45.6	45.6	45.7	45.8	45.9
Current smoking (%)	33.1	29.0	27.7	26.6	25.2
Body mass index*	24.1	24.1	24.0	24.0	23.8
Physical activity (hours/week)	2.8	2.9	3.1	3.1	3.2
Any weight loss in prior 2 years (%)	29.7	28.6	29.4	29.1	29.8
Total energy intake (kcal/day)	1,578	1,556	1,566	1,570	1,584
Oral contraceptive use (%)	52.2	51.3	50.6	50.0	49.7
Use of hormone replacement therapy† (%)	14.0	13.5	13.6	13.7	13.4
Use of aspirin (%)	46.5	47.0	46.8	46.2	44.3
Use of thiazide diuretics (%)	8.6	8.2	8.4	8.0	7.6
Alcohol intake (g/day)	8.4	7.1	6.2	5.8	5.1
Caffeine intake (g/day)	377	388	400	405	393
Dietary intake (g/day)					
Saturated fat	32.2	29.5	27.9	26.4	23.9
Trans fat	4.1	4.1	4.1	4.0	3.8
Polyunsaturated fat	8.5	9.0	9.3	9.7	10.5
Monounsaturated fat	32.5	29.8	28.4	27.1	25.6
Carbohydrate	132	148	157	164	173
Fiber	9.2	11.6	13.1	14.5	17.4
Foods consumed (no. of servings/day)					
Red meat	1.8	1.5	1.4	1.3	1.0
Chicken and turkey	0.2	0.2	0.3	0.3	0.4
Fish	0.1	0.2	0.2	0.2	0.2
Fruits	1.6	2.0	2.1	2.3	2.4
Vegetables	1.4	1.7	1.9	2.1	2.6

* Weight (kg)/height (m)^2.
† Only postmenopausal women were included.

Assessment of diet

Dietary information was derived from semiquantitative food frequency questionnaires (15) administered in 1980, 1984, 1986, 1990, 1994, and 1998. Participants were asked to indicate their frequency of consumption, on average, of typical servings of selected foods during the previous year. There were nine possible response options, ranging from never or less than once per month to six or more times per day. We computed nutrient scores by multiplying the frequency of consumption of each unit of food from the semiquantitative food frequency questionnaire by the nutrient content of the specified portion according to food composition tables obtained from the US Department of Agriculture (16) and other sources.

A detailed description of the semiquantitative food frequency questionnaire and the procedures used for calculating nutrient intake, as well as data on reproducibility and validity, has been provided elsewhere (15). All nutrient intakes were adjusted for total energy intake using regression analysis. This approach is based on the concept that the composition of the diet, independent of total energy intake, is most relevant to dietary recommendations (15). In validation studies, the Pearson correlation coefficients for correlations between energy-adjusted protein intakes recorded on the 61- and 116-item food frequency questionnaires compared with four 1-week diet records were 0.44 and 0.52, respectively. The correlation coefficient for energy-adjusted protein intakes assessed by the two questionnaires administered 4 years apart was 0.53.

Ascertainment of cases of cholecystectomy

We inquired about the occurrence and date of cholecystectomy on each biennial questionnaire starting in 1980. A validation study of the self-report was conducted in a random sample of 50 nurses who reported undergoing cholecystectomy in 1982. Forty-three out of 50 participants responded.

TABLE 3. Correlations among energy-adjusted protein intake and intakes of fat, carbohydrate, and dietary fiber at baseline, Nurses' Health Study, 1980*

Dietary variable	Spearman correlation coefficient		
	Total protein intake	Animal protein intake	Vegetable protein intake
Carbohydrates	–0.42	–0.49	0.39
Fats			
Total fat	0.16	0.24	–0.34
Saturated fat	0.2	0.32	–0.46
Polyunsaturated fat	–0.12	–0.18	0.24
Monounsaturated fat	0.19	0.27	–0.35
Trans fat	–0.16	–0.12	–0.07
Fiber	0.07	–0.10	0.62

* All *p*'s < 0.001.

Of these, all reiterated their earlier report, and the diagnosis of gallstone disease was confirmed in all 36 participants for whom medical records could be obtained. Cholecystectomy was used as an endpoint mainly because gallstone disease is mostly treated surgically (17) and because women are more likely to accurately report the occurrence and timing of a surgical procedure than the occurrence of untreated gallstones. In addition, symptomatic gallstones are the main indication for cholecystectomy.

Data analysis

We calculated person-time of follow-up for each participant from the date of return of the 1980 questionnaire to the date of cholecystectomy, cancer, last questionnaire return, death, or the end of the study period in 2000, whichever came first. Women were categorized into quintiles of dietary protein intake. We calculated incidence rates by dividing the number of events by the number of person-years of follow-up in each quintile. Relative risks were calculated as the incidence rate of cholecystectomy among women in different categories of exposure compared with the incidence rate among women in the reference category, with adjustment for age in 5-year categories. The incidence of cholecystectomy was examined in relation to the cumulative average of exposure variables from all available questionnaires up to the start of each 2-year follow-up interval, using methods for repeated measurements (18). Multivariate relative risks were computed using Cox proportional hazards regression (19). In multivariate analyses, we simultaneously adjusted for the following known or suspected confounding variables: time period, age, body mass index (weight (kg)/height (m)^2), weight change in the previous 2-year interval, physical activity, parity, use of oral contraceptives, postmenopausal use of hormones, pack-years of smoking, use of thiazide diuretics, use of nonsteroidal antiinflammatory drugs, and intakes of alcohol, coffee, dietary fiber, and total energy. We additionally included specific types of fat (saturated, monounsaturated, polyunsaturated, and *trans* fats) in the models so that the effects of protein intake were compared with those of carbohydrates. We conducted tests for linear trend across increasing quintiles of protein intake by assigning the median value to each quintile and treating these values as a single continuous variable. All relative risks are presented with 95 percent confidence intervals, and all reported *p* values are two-sided. All analyses were performed with Statistical Analysis System software, release 8.2 (SAS Institute, Inc., Cary, North Carolina).

RESULTS

At baseline in 1980, women with a higher intake of animal protein (table 1) tended to consume less alcohol, coffee, carbohydrate, and polyunsaturated fat and tended to be more physically active, but were less likely to be current smokers. Women with a higher vegetable protein intake (table 2) tended to consume less alcohol and saturated and monounsaturated fats and tended to be more physically active, but were less likely to be current smokers. Intakes of animal and vegetable protein were inversely correlated ($r = -0.34$). Total protein intake was modestly correlated with intake of carbohydrates and slightly correlated with intakes of total fat and specific types of fat (table 3). Intake of animal protein was negatively correlated with fiber intake, whereas intake of vegetable protein was positively correlated with fiber intake.

During 1,393,255 person-years of follow-up from 1980 to 2000, we recorded 7,831 cases of cholecystectomy. After adjustment for age and smoking, the estimated relative risk for women in the highest quintile of energy-adjusted dietary total protein intake compared with those in the lowest quintile was 1.27 (95 percent confidence interval (CI): 1.18, 1.37; *p* for trend < 0.0001) (table 4). This association became nonsignificant after further adjustment for other suspected or known risk factors for gallstones. In an analysis that included age, body mass index, recent weight change, parity, use of oral contraceptives, hormone replacement therapy, physical activity, pack-years of smoking, use of thiazide diuretics, use of nonsteroidal antiinflammatory drugs, and intakes of alcohol, coffee, and total energy, the relative risk for the highest quintile of dietary protein intake compared with the lowest was 0.98 (95 percent CI: 0.91, 1.05; *p* for trend = 0.96). Additional adjustment for intakes of saturated fat, monounsaturated fat, polyunsaturated fat, and *trans* fat did not materially alter the relative risk (relative risk = 1.00, 95 percent CI: 0.93, 1.08).

We further examined the relative risks separately for intakes of animal protein and vegetable protein. For animal protein intake, after adjustment for age and smoking, the relative risk for women in the highest quintile compared with those in the lowest was 1.39 (95 percent CI: 1.30, 1.50; *p* for trend < 0.0001). The association was attenuated and became nonsignificant after further adjustment for other risk factors and specific fats (model 3). When extreme quintiles of animal protein intake were compared, the relative risk was 1.07 (95 percent CI: 0.98, 1.15; *p* for trend = 0.08). Additional adjustment for vegetable protein intake slightly attenuated the association further (relative risk = 1.03, 95 percent CI: 0.95, 1.11; *p* for trend = 0.08).

For vegetable protein intake, the age- and smoking-adjusted relative risk for women in the highest quintile

TABLE 4. Adjusted relative risk of cholecystectomy according to quintile of energy-adjusted intake of dietary protein among US women, Nurses' Health Study, 1980–2000

	Quintile of dietary protein intake					*p* for trend
	1 (lowest)	2	3	4	5 (highest)	
			Total protein intake			
No. of cases	1,309	1,483	1,561	1,748	1,730	
Median intake (g/day)	60.0	68.2	74.0	79.9	89.9	
Model 1*: RR†	1.00	1.01 (0.93, 1.08)‡	1.05 (0.97, 1.13)	1.19 (1.11, 1.28)	1.27 (1.18, 1.37)	<0.0001
Model 2§: Multivariate RR	1.00	0.97 (0.90, 1.04)	0.95 (0.88, 1.03)	1.02 (0.95, 1.09)	0.98 (0.91, 1.05)	0.96
Model 3¶: Multivariate RR	1.00	0.97 (0.90, 1.05)	0.96 (0.89, 1.04)	1.03 (0.96, 1.12)	1.00 (0.93, 1.08)	0.46
			Animal protein intake			
No. of cases	1,354	1,515	1,623	1,677	1,662	
Median intake (g/day)	42.6	50.8	56.6	63.0	74.0	
Model 1*: RR	1.00	1.08 (1.00, 1.16)	1.17 (1.09, 1.26)	1.26 (1.17, 1.35)	1.39 (1.30, 1.50)	<0.0001
Model 2§: Multivariate RR	1.00	1.02 (0.94, 1.09)	1.03 (0.96, 1.11)	1.05 (0.97, 1.12)	1.04 (0.97, 1.12)	0.2
Model 3¶: Multivariate RR	1.00	1.02 (0.95, 1.10)	1.04 (0.97, 1.12)	1.06 (0.98, 1.14)	1.07 (0.98, 1.15)	0.08
Further adjustment for vegetable protein intake	1.00	1.01 (0.94, 1.09)	1.03 (0.95, 1.11)	1.04 (0.96, 1.12)	1.03 (0.95, 1.11)	0.08
			Vegetable protein intake			
No. of cases	1,274	1,527	1,621	1,809	1,600	
Median intake (g/day)	11.6	14.9	17.0	18.9	21.8	
Model 1*: RR	1.00	0.94 (0.87, 1.01)	0.88 (0.81, 0.95)	0.90 (0.83, 0.98)	0.76 (0.71, 0.83)	<0.0001
Model 2§: Multivariate RR	1.00	0.91 (0.84, 0.99)	0.84 (0.77, 0.92)	0.86 (0.79, 0.95)	0.77 (0.70, 0.86)	<0.0001
Model 3¶: Multivariate RR	1.00	0.91 (0.84, 0.99)	0.84 (0.77, 0.93)	0.87 (0.79, 0.96)	0.79 (0.71, 0.88)	<0.0001
Further adjustment for animal protein intake	1.00	0.92 (0.84, 1.00)	0.85 (0.77, 0.93)	0.88 (0.79, 0.97)	0.80 (0.72, 0.90)	<0.0001

* Model 1: Relative risks were adjusted for age (1-year categories) and pack-years of smoking (0, 1–9, 10–24, 25–44, 45–64, or ≥65 pack-years).

† RR, relative risk.

‡ Numbers in parentheses, 95% confidence interval.

§ Model 2: The multivariate model included the following: age (1-year categories), time period (1980–1982, 1982–1984, 1984–1986, 1986–1988, 1988–1990, 1990–1992, 1992–1994, 1994–1996, 1996–1998, or 1998–2000), body mass index (weight (kg)/height (m)^2) at the beginning of each 2-year follow-up interval (<20.00, 20.00–22.49, 22.50–24.99, 25.00–27.49, 27.50–29.99, 30.00–32.49, 32.50–34.99, 35.00–37.49, 37.50–39.99, or ≥40), weight change in the previous 2 years (weight loss of ≥10 pounds (>4.5 kg), weight loss of 5.0–9.9 pounds (2.3–4.5 kg), weight maintenance at ±4.9 pounds (±2.2 kg), weight gain of 5.0–9.9 pounds (2.3–4.5 kg), or weight gain of ≥10 pounds (>4.5 kg)), parity (0, 1, 2–3, or ≥4 births), use of oral contraceptives (ever vs. never), hormone replacement therapy (premenopausal, postmenopausal without hormone replacement therapy, postmenopausal with past hormone replacement therapy, or postmenopausal with current hormone replacement therapy), physical activity (quintiles), energy-adjusted intake of dietary fiber (quintiles), pack-years of smoking (0, 1–9, 10–24, 25–44, 45–64, or ≥65 pack-years), use of thiazide diuretics (yes or no), history of diabetes mellitus (yes or no), use of nonsteroidal antiinflammatory drugs (0, 1–6, or ≥7 times per week or dose unknown), alcohol drinking (0, 0.1–4.9, 5.0–14.9, 15.0–29.9, or ≥30.0 g/day), coffee consumption (0, 1, 2–3, or ≥4 cups/day), and total energy intake (quintiles).

¶ Model 3: The model included all variables listed above for model 2, with additional adjustment for specific fats (saturated fat, polyunsaturated fat, monounsaturated fat, and *trans* fat) in quintiles.

compared with those in the lowest was 0.76 (95 percent CI: 0.71, 0.83; p for trend < 0.0001). The relative risk remained significant after further adjustment for other risk factors and specific fats. When extreme quintiles of vegetable protein intake were compared, the relative risk was 0.79 (95 percent CI: 0.71, 0.88; p for trend < 0.0001). The relative risk was not materially changed and the inverse association remained significant after additional adjustment for animal protein (relative risk = 0.80, 95 percent CI: 0.72, 0.90; p for trend < 0.0001).

We also performed multivariate analyses using deciles of total protein, animal protein, and vegetable protein consumption. After adjustment for potential risk factors and specific fats, women in the highest decile of total protein intake had a relative risk of 0.97 (95 percent CI: 0.87, 1.08; p for trend = 0.46) compared with those in the lowest decile.

TABLE 5. Multivariate relative risk of cholecystectomy according to quintile of intake of vegetable protein among US women, Nurses' Health Study, 1980–2000*

Variable	Quintile of vegetable protein intake					*p* for trend
	1 (lowest)	2	3	4	5 (highest)	
Age (years)						
≤60	1.0	0.91	0.84	0.84	0.75	<0.0001
>60	1.0	0.94	0.89	0.97	0.93	0.02
Body mass index†						
<25	1.0	0.97	0.83	0.88	0.73	0.0002
25–30	1.0	0.94	0.89	0.91	0.82	0.06
>30	1.0	0.80	0.79	0.74	0.78	0.06
Current smoking						
No	1.0	0.93	0.88	0.92	0.81	0.003
Yes	1.0	0.88	0.74	0.75	0.76	0.008
Fiber intake‡						
Low	1.0	0.90	0.81	0.86	0.77	0.0003
High	1.0	0.98	0.96	0.96	0.87	0.01
Coffee consumption						
No	1.0	0.97	0.84	0.86	0.76	0.0005
Yes	1.0	0.85	0.83	0.85	0.79	0.01

* The multivariate model included the same covariates as in model 3 in table 3. The variable used for stratification was excluded from the model.

† Weight (kg)/height (m)^2.

‡ The median value was used as the cutoff point.

For animal protein intake, the relative risk was 1.00 (95 percent CI: 0.90, 1.12; *p* for trend = 0.08) when extreme deciles were compared. In contrast, women in the highest decile of vegetable protein intake had a significantly reduced relative risk of 0.74 (95 percent CI: 0.64, 0.86; *p* for trend < 0.0001) in comparison with those in the lowest decile.

To address the effect of longer-term protein intake, we evaluated the association between baseline vegetable protein intake and risk of cholecystectomy. Compared with women in the lowest quintile, women in the highest quintile of vegetable protein intake had a multivariate relative risk of 0.89 (95 percent CI: 0.81, 0.98; *p* for trend = 0.005). The inverse association, though slightly attenuated, was similar to that obtained when cumulative average updated exposure information was used.

To examine whether the relative risk of vegetable protein intake might be modified by other risk factors, we repeated the multivariate analyses within subgroups of potentially confounding variables (table 5). The inverse associations between vegetable protein intake and risk of cholecystectomy were seen in virtually all subgroups, though they were not always statistically significant, and we found no apparent modification of the relation.

To examine the possibility that latent gallstone symptoms caused a change in diet, thereby biasing the results, we conducted an analysis excluding all cases that occurred during the first 4-year follow-up period and relating vegetable protein intake in 1980 to the incidence of cholecystectomy in 1984–2000. Compared with women in the lowest quintile, women in the highest quintile of vegetable protein intake had a multivariate relative risk of 0.82 (95 percent CI: 0.74, 0.92; *p* for trend = 0.001). Additional exclusion of women who did not have a routine medical check-up between 1986 and 1988 did not alter the relation materially; when extreme quintiles were compared, the multivariate relative risk was 0.81 (95 percent CI: 0.72, 0.92; *p* for trend = 0.001).

DISCUSSION

In this large, prospective cohort study, we found that a high intake of vegetable protein was associated with a significantly reduced risk of cholecystectomy, whereas neither animal protein intake nor total protein intake was related to risk. Our detailed analyses took into consideration the differences in lifestyle and other dietary factors for which we had information. The inverse association between vegetable protein intake and risk of cholecystectomy could not be explained by these known or suspected risk factors.

In a crossover study in humans (6), subjects were randomly assigned to either a low-protein, high-carbohydrate diet or a high-protein, low-carbohydrate diet for 4–5 weeks. Intakes of dietary fat and fiber were kept constant. Replacement of carbohydrate with protein significantly reduced triglyceride concentrations by 23 percent and increased high density lipoprotein cholesterol concentrations by 12 percent. In addition, consumption of vegetable protein rather than animal protein has been consistently demonstrated to decrease serum concentrations of triglycerides in controlled trials (20).

There is clear evidence in laboratory animals that dietary protein reduces gallstone formation, though the mechanisms are not clearly defined. In hamsters, dietary vegetable protein markedly reduced the formation of cholesterol gallstones (21); 58 percent of the hamsters developed gallstones when their diet contained casein, an animal protein, as compared with 14 percent of the hamsters that were fed on a diet containing soybean protein. Furthermore, once cholesterol gallstones were established in the hamsters, significant dissolution of gallstones occurred with administration of a soybean protein diet. Similar findings of decreased cholesterol gallstone formation were also demonstrated in rabbits fed on a vegetable protein diet compared with those fed on an animal protein diet (22). In a recent study, vegetable protein exerted an inhibitory effect on biliary cholesterol crystallization, which is the determinant step in gallstone genesis (10). These results imply that substituting vegetable protein for part of the animal protein supply or other macronutrients in Western diets could be effective in primary or secondary prevention of gallstones at the earliest stage of crystal formation in humans.

Epidemiologic or clinical studies regarding dietary protein intake and risk of gallstone disease are sparse. In a case-control study, protein intake was not associated with risk of incident gallstones diagnosed by ultrasonography (23). In a cross-sectional epidemiologic survey carried out in Italy, a low protein intake appeared to be protective against gallstones in males (24). The incidence of gallstones in autopsy statistics, with international comparisons, suggested that a hypocaloric diet associated with a low intake of protein, mostly of vegetable origin, was protective against cholelithiasis (25). A 48-hour dietary recall survey among cholelithiasis subjects and controls found that the female cholelithiasis group consumed less protein than the female control group (26). These results have been limited by a lack of long-term dietary information, nonvalidated assessment of nutrients, a suboptimal study design, or inadequate control for confounding.

In this cohort, the possibility of misclassification might be of concern, because information on nutrient intake was collected by self-report. Random within-person variation could attenuate any true association of interest, but the semi-quantitative food frequency questionnaire was designed to minimize this error by assessing average long-term dietary intake during the successive follow-up periods. These repeated measurements took into account possible changes in eating behavior and food composition over time and reduced random variation in reporting. Reporting of dietary factors has been extensively validated in subsamples of the Nurses' Health Study cohort (27). Moreover, our prospective study design precluded bias attributable to differential recall of protein consumption by women with and without cholecystectomy. Our findings are probably not caused by underascertainment of cholecystectomy cases, because this would not bias the observed risks (28).

We were concerned about the possibility of bias due to latent gallstone disease. However, such bias is unlikely to have influenced our results substantially, because the inverse associations persisted after we incorporated a lag period of 4 years between vegetable protein intake at baseline and subsequent cholecystectomy or when we excluded women without regular checkups.

In summary, these results suggest that higher long-term intake of vegetable protein in the context of an energy-balanced diet can reduce the risk of cholecystectomy in women. This conclusion is supported by evidence of a dose-response relation and the consistency of the findings with results from experimental studies.

ACKNOWLEDGMENTS

This study was supported by research grants CA87969 and DK46200 from the National Institutes of Health.

The authors thank Gary Chase, Karen Corsano, Lisa Dunn, Barbara Egan, Lori Ward, Mary Louie, Al Wing, and Laura Sampson for their expert assistance.

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